bio-protocol Preprint

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Cell cycle analysis with BrdU and propidium iodide

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An abbreviated version of this protocol was published in eLIFE in Aug 2014

Mismatch repair deficiency endows tumors with a unique mutation signature and sensitivity to DNA double-strand breaks

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Detailed protocol

1. Seed 300,000 cells/well in 6-well plates and treat with 26uM olaparib, 300nM mitomycin C, 30nM camptothecin or placebo.
2. After 24 hours, incubate cells with BrdU at a final concentration of 10uM for 90 minutes.
3. Trypsinize cells for 5 min.
4. Add 2mL medium to stop trypsinization
5. Centrifuge @400g, 5 min, 4°C
6. Resuspend the pellet in 500 uL ice-cold PBS.
7. Slowly add 2mL 100% ice-cold ethanol is while mixing the resuspension
8. Incubate for 5 minutes at room temperature to allow fixation.
9. Centrifuge @400g, 5 min
10. Resuspend in 2mL PBS for washing
11. Centrifuge @400g, 5 min, 4°C
12. Resuspend in 2mL PBS for washing
13. Centrifuge @400g, 5 min, 4°C
14. Resuspend in 2M HCl/0.05% Tween20 in PBS for 30 minutes to denature DNA
15. Centrifuge @400g, 5 min, 4°C
16. Resuspend in 0.5% normal goat serum/0.05% Tween20 in PBS, incubate for 10 minutes at room temperature.
17. Add FITC-conjugated anti-BrdU antibody diluted 1:10 in 0.5% NGS/0.05% Tween20 in PBS at room temperature.
18. Centrifuge @400g, 5 min, 4°C
19. Wash away antibody by resuspending the pellet with 0.05% Tween20 in PBS
20. Centrifuge @400g, 5 min, 4°C
21. resuspend pellet in 25 ug/mL propidium iodide containing 0.1 mg/mL RNase A.
22. Measure fluorescence on a BD Biosciences FACSVerse flow cytometer
23. Model the cell cycle data using FlowJo software. The fraction of cells in S-phase, G2/M and G1 are determined as described by Watson et al. (1987).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zhao, H. , Thienpont, B. and Lambrechts, D. (2022). Cell cycle analysis with BrdU and propidium iodide. Bio-protocol Preprint. bio-protocol.org/prep1821.
2. Zhao, H., Thienpont, B., Yesilyurt, B. T., Moisse, M., Reumers, J., Coenegrachts, L., Sagaert, X., Schrauwen, S., Smeets, D., Matthijs, G., Aerts, S., Cools, J., Metcalf, A., Spurdle, A., Amant, F. and Lambrechts, D.(2014). Mismatch repair deficiency endows tumors with a unique mutation signature and sensitivity to DNA double-strand breaks. eLIFE. DOI: 10.7554/eLife.02725